

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
12 February 2004 (12.02.2004)

PCT

(10) International Publication Number
WO 2004/012709 A1

(51) International Patent Classification⁷: **A61K 9/127**,
48/00

(21) International Application Number:
PCT/CN2003/000634

(22) International Filing Date: 6 August 2003 (06.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/401,686 6 August 2002 (06.08.2002) US
60/425,825 13 November 2002 (13.11.2002) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/012709 A1

(54) Title: COCHLEATES WITHOUT METAL CATIONS AS THE BRIDGING AGENTS

(57) Abstract: This invention provides cochleate and nano-cochleate systems wherein the agents bridging lipid bilayer are organic multi-valent cations. This invention also provides a method for preparing the cochleate system comprising direct cochleation and hydrogel isolated procedure. The preparation method comprises using the charge ration between the bridging agents and lipids to control the particle sizes. This cochleate or nano-cochleate system may be used for microencapsulation and delivery of therapeutics wherein the therapeutic agents are loaded in the cochleate structure as the bridging agents between lipid bilayers. Finally, this invention provides other uses of this new cochleate and nano-cochleate systems.

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COCHLEATES WITHOUT METAL CATIONS AS THE BRIDGING AGENTS**CROSS-REFERENCE TO RELATED APPLICATION**

- 5 This application claims priority of U.S. Serial No. 60/401,686 filed on August 6, 2002, and U.S. Serial No. 60/425,825 filed on November 13, 2002, the content of which are incorporated here into this application.

Throughout this application, various references are referred to and disclosures of these
10 publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

FIELD OF THE INVENTION

- 15 The present invention demonstrates a novel phospholipids composition and its application in delivering various therapeutic agents to which tissue and/or membranes are impermeable. This composition comprises negatively charged lipid bilayers which interact with organic multicationic cations to roll up forming a cylindrical multi-layer structure.

20 **BACKGROUND OF THE INVENTION**

- While the progress of biotechnology has brought more and more biological therapeutics to clinical applications, development of appropriate dosage forms for these agents are far behind the pace of development of the agents. Owing to their tissue impermeability and in vivo
25 instability, most of biological therapeutic agents are given by frequent injection [1], that resulted in poor patient compliance. Advanced drug delivery systems for biological agents have attracted considerable research efforts in past years [2].

- Among various drug delivery routes, oral delivery is by far easiest and most convenient way for drug administration, especially when repeated dose and long therapeutic period are necessary. Many approaches have been reported for oral delivery of tissue impermeable drugs [3]. Strategies to improve oral absorption may be divided to i) converting a drug to lipophilic pro-drug, ii) conjugating a drug with lipophilic moieties, and iii) encapsulating a drug into particulate systems [3]. Particulate systems may offer good protection of delicate biological agents with no need of chemical modification of the molecules selves. However, absorption of particles by intestine is generally less than 1% [3].
- Because of the structure similarity of liposomes (phospholipid bilayer vesicles) to cellular membranes, the material had once been regarded as an ideal system for delivering therapeutics and attracted considerable research effort since its discovery roughly four decades ago [4]. However, their physicochemical and biological instability retarded the success in practical application of liposomes in drug therapy. There are only limited liposome-based formulations are commercially available despite the R & D efforts remunerated [5]. To overcome this stability problem, several alternative lipid bilayer systems have been reported, namely, stealth™ liposomes [5], polymerized liposomes [6], polyethylene glycol coated liposomes [7], lipo-beads [8], and cochleates [9].
- Cochleates are spiral rolls formed of negatively charged phospholipid bilayers which are rolled up through the interaction with multivalent counter ions (Ca^{2+} or Zn^{2+}) as the bridging agents between the bilayers [9]. As a particulate system, cochleates possess unique properties that they offer superior mechanical stability and better protection for drugs encapsulated than liposomes due to their solid matrix, yet they remain the phospholipid bilayer structure. These solid particles are so flexible that they can readily convert to liposomes by extracting the bridging counter ions out of the inter bilayer spaces. Such unique properties have made cochleates an ideal system for delivering insoluble ingredients which can be loaded in the matrix of a phospholipid bilayer but avoid the instability problem of liposomes [10].

Recently, the present inventor has patented another technology that demonstrated structure, preparation and application of nanometer-sized cochleates (nanocochleates) [11]. These size-reduced cochleates showed capability for oral delivery of Amphoterecin B (AmB), a hydrophobic drug currently administrated through IV injection in the form of liposomes or micelles. Oral availability of AmB achieved by nanocochleates encourage the inventor to design a new cochleate system by which highly charged and membrane-impermeable therapeutics may be encapsulated and delivered orally.

10 In the case that cochleates are used as drug carriers, hydrophobic drug molecules are incorporated in the matrix of phospholipid bilayer prior to cochleation (formation of cochleates by addition of metal cations). Drug loading capacity is limited by how much drug can be "dissolved" in the lipid matrix without destroying its bilayer structure. This structure limits application of cochleates to delivery of hydrophobic molecules.

15 It has been reported that cochleates were used to delivery DNA and protein vaccines [12,13]. The authors suggested that these hydrophilic macromolecules were incorporated in the cochleate structure. However, there was no solid evidence presented by the authors. The only experimental observation was that the concentration of DNA or proteins in the supernatant was reduced after addition of calcium ions. Since the multivalent ions (Ca^{2+} or Zn^{2+}) used in cochleate formation can also complexed with proteins and DNA (both are charged polymers) through ionic interaction, the likelihood is that these macromolecules interacted with Ca^{2+} or Zn^{2+} and were merely precipitated at the same time when cochleates were forming. Owing to the size and hydrophilic nature of these macromolecules, encapsulation of proteins and DNA into the lipid bilayer matrix or the inter-bilayer space is unlikely.

In the present invention, a new approach that ensures hydrophilic drug being loaded in the inter-bilayer space of cochleates and nanocochleates is demonstrated.

SUMMARY OF THE INVENTION

5

The present invention demonstrates a new type of cochleates and nano-cochleates that allow charged, soluble but tissue-impermeable molecules, including relatively small therapeutic peptides, be encapsulated in the inter-bilayer space and delivered cross tissue-membrane. Cochleates and nano-cochleates are phospholipid-calcium (or zinc) precipitates that are
10 formed by calcium (or zinc) induced fusion of unilamellar liposomes into large lipid bilayer sheets which then fold spirally into cylinders. The new cochleates and nano-cochleates differ from conventional systems in that i) the fusion of unilamellar liposomes is no longer induced by Ca^{2+} , Zn^{2+} or other metal ions but by the molecules to be encapsulated (See Figure 1); ii) charged, hydrophilic and tissue impermeable drugs can be encapsulated in the structure with
15 improved loading capacity. Since no additional metal cations (such as Ca^{2+} or Zn^{2+}) are existing during the new cochelation process, there is no such possibility that the molecules to be encapsulated are precipitated outside of the cochleate structure as in the conventional cochelation.

20 On other hand, the new cochleates and nano-cochleates showed some similarities in physical chemical properties and drug delivery functions as the conventional systems. The cylindrical structure could open-up and converted to liposomes upon adding a cation carrier, such as EDTA. In addition, the new system showed the ability to deliver encapsulated ingredients across cell membranes by fusion with the membrane (See Figure 2).

25

Organic cations with various sizes (2,3,4,5,-tetraaminopyrimidine, tobramycin, and polylysine) were examined in the present invention, and the cylindrical cochleate structure was observed

in either case. These results suggest a wide flexibility in formation of cochleates with organic cations as the bridging agents between the lipid bilayers.

This invention offers a simplified method to prepare nano-cochleates. When poly-cations
5 (such as polypeptides with net charge over 5) were used, nano-cochleates were easily prepared by adding the polycations directly into the liposomal suspension, without using complicated hydrogel-isolation technique.

DETAILED DESCRIPTION OF THE FIGURES

10

Figure 1. Schematic description of complexation of phospholipids bilayers with Ca^{2+} , and with organic cations.

15

Figure 2. Schematic description of fusion of cochleates formed by interaction with drug molecules which function as the bridging agent between phospholipids bilayers. The loaded drug molecules are delivered across cell membranes due to fusion of cochleates with the cell membrane.

20

Figure 3. Microscopic image of cochelates formed by complexation with 2,3,5,6-tetraaminopyrimidine as the bridging agent. A: before treatment with EDTA; B: after treatment with EDTA.

25

Figure 4. Microscopic image of nano-cochelates formed by complexation with 2,3,5,6-tetraaminopyrimidine as the bridging agent. A: before treatment with EDTA; B: after treatment with EDTA.

Figure 5. Microscopic image of cochelates formed by complexation with tobramycin as the bridging agent. A: before treatment with EDTA; B: after treatment with EDTA.

Figure 6. Microscopic image of nano-cochelates formed by complexation with tobramycin as the bridging agent. A: before treatment with EDTA; B: after treatment with EDTA.

Figure 7. Distribution of dynamic sizes of nano-cochleates formed by complexation with tobramycin.

Figure 8. Microscopic image of cochelates formed by complexation with polylysine as the bridging agent. A: before treatment with EDTA; B: after treatment with EDTA.

Figure 9. Antibiotic activity of tobramycin formulated in solution, cochleates and nano-cochleates. The drug of various doses were added to E.Coli prior to incubation at 37 °C, followed by counting the colonies.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a new cochleate system and a nano-cochleate system for which the agents that bridge lipid bilayers together to form a multi-layer structure are organic multi-valent cations. As used herein, the new cochleates systems is defined as a spiral phospholipids bilayer that rolled up by complexation with organic multi-valent cations which bring two surfaces of charged lipid bilayers together through ionic bonds (See figure 1). The multi-bilayer systems formed by interaction with the organic cations may or may not form a cylindrical shape.

The new systems can allow charged and hydrophilic therapeutics, such as peptides, be microencapsulated into the cochleate structure while conventional cochleates cannot. On the other hand, however, the new systems showed the similar properties observed from conventional cochleates such as conversion back to liposomes when treated with cation carriers, and the ability to deliver drugs across tissue membranes. These properties (loading hydrophilic drugs and delivery across membrane) make the new systems promising to deliver peptides orally.

The cochleate and nano-cochleate systems disclosed herein can be used for microencapsulation and delivery of therapeutics wherein the therapeutics agents are loaded in the cochleate structure as the bridging agents between lipid bilayers.

The therapeutics includes but is not limited to peptides, poly-amino acids, nucleotides and hydrophilic chemical drugs which possess two or more net charges.

In an embodiment, the above-described cochleate systems are used for oral delivery of peptides, polyamino acids, nucleotides and hydrophilic chemical drugs which possess more than two net positive charges. Other drugs may be used. An ordinary skilled artisan may use the drugs exemplified herein or the guidelines provided in other drugs.

In another embodiment, the delivery of therapeutics is through inhalation.

This invention also provides a method of preparing the new cochleate systems comprising direct cochleation [9], hydrogel-isolated cochleation [11], and size-controlled cochleation using poly-cations (See Example 5). Organic cations can be added to a suspension of unilamellar liposomes directly with stirring or vortex, or added to an polymer aqueous two-phase system for which liposomes are partitioned in the dispersed phases and isolated within each droplet [11].

Another advantage of this invention is that nano-cochleates can be prepared without using the complicated hydrogel-isolation technique [11]. For those polycations which possess multiple net charges (usually more than 5), the sizes of cochleates formed can be controlled by the charge ratio of the polycations over liposomes. By increasing the polycations over stoichiometrical amount, nano-cochleates can be formed by adding the polycations directly to the liposomal suspension (Example 5). Due to their multiple net charges and long chain, polycations may be partially associated with the lipids of opposite charge, leaving some charged species dangling at the cochleate surfaces. This invention offers a significantly simplified method to prepare nano-cochleates.

The invention will be better understood by reference to the examples which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

EXAMPLES

Example 1: Preparation of cochleates with 2,3,5,6-tetraaminopyrimidine sulfate

A multivalent organic cation, 2,4,5,6-tetraaminopyrimidine sulfate (TAS), was dissolved in water with concentration of 10.5 mM and pH of 2 (adjusted with HCl). A suspension of small unilamellar liposomes (SUV) was prepared by suspending dioleoyl phosphatidyl serine (DOPS) in water, followed by sonication in a N₂ atmosphere. The lipid-water suspension looked milky at beginning, but turned to be clear (with slightly blue tint) as sonication proceeded. The sample was examined using an optical microscope and no liposome was observed, indicating that liposomes are smaller than 1 micron.

To prepare cochleates, the TAS solution was added to the liposome suspension drop-wise under magnetic stirring until precipitation occurred. The precipitates were examined using a microscope, and the microscopic image showed that the lipids formed needle shape structures (See Figure 3A). Other organic cationic molecules, such as antibiotics and polypeptides, can
5 also be used to form cochleate structure.

To examine properties of the new cochleates, a drop of EDTA (200 mM, pH 8.5) was added to the precipitates loaded on a microscope slight and observed under the microscope. As shown in Figure 3B the needle shape precipitates opened up and converted to giant liposomes. This
10 is a typical property of conventional cochleates. The results in Figure 3A and 3B indicate that organic multivalent cations interact with liposomes (made of DOPS) to form cochleates as those formed with Ca^{2+} and Zn^{2+} .

Example 2: Preparation of nano-cochleates with 2,3,5,6-tetraaminopyrimidine sulfate.

Nano-sized cochleates can be prepared with 2,3,5,6-tetraaminopyrimidine sulfate using hydrogel-
15 isolated methods that the inventor has patented previously [11]. In brief, a liposome suspension prepared as in Example 1 was added into a dextran solution (5-25%) with lipid content of 0.2-2%. This suspension was then dispersed into a polyethylene glycol (PEG) solution (5-25%) and well stirred. The solutions of dextran and PEG were immiscible and formed an aqueous two-phase system. The TAS solution prepared as in Example 1 was
20 added drop-wise to the aqueous two-phase system under stirring with the charge of the organic cations was more than that of the lipids. The aqueous two-phase system was stirred for additional 10 to 60 min, and then the cochleates formed were recovered by rinsing the dextran and PEG away using sufficient amount of water (by which dextran and PEG were all dissolved in one phase), followed by centrifugation. Figure 4A show a microscopic image of
25 recovered cochleates. Needle structure was not detectable by optical microscope due to the particle size. A laser scattering measurement showed that the cochleate sizes were in sub-

micron. Figure 4B shows the microscopic image of nano-cochleates after treatment with EDTA. Liposomes formed from nano-cochleates are much smaller than those from the cochleates formed without hydrogel-isolation (compare Figure 3B with Figure 4B). The similar result that EDTA treatment of nano-cochleates only generated small liposomes was reported
5 in the inventor's previous invention of calcium induced nano-cochleates [11]. The particle size distribution of the nano-cochleates was measured using a Nicomp submicron particle sizer, showing the mean dynamic size was around 400 nm.

Example 3. Preparation of cochleates and nano-cochleates with tobramycin

Cochleates and nano-cochleates were prepared by repeating the experimental procedure in
10 example 1 and 2 using a drug, tobramycin chloride, as the bridging agent instead of TAS. Tobramycin is an antibiotic, soluble in water in salt form and administered by injection. The molecule has molecular weight of 467 and 5 amino groups.

To prepare cochleates, a solution of tobramycin was prepared by dissolving 100 mg tobramycin with 100 ml water. Prior to cochleation, the solution was divided to several parts
15 with pH adjusted to 1.2, 2.5, 3.5 and 5, respectively. These drug solutions were added dropwise to liposome solutions prepared as in Example 1, respectively. Visible precipitates were formed for the samples treated with tobramycin solution with pH of 1.2 and 2.5, suggesting that sufficient ionization of the amino groups of tobramycin is required. The formed cochleates and their response to EDTA were examined using an optical microscope. The
20 images were shown in Figure 5A and 5B, respectively. For the precipitates showed needle shapes (Figure 5A) before treatment with EDTA, and converted to giant liposomes when EDTA was added (Figure 5B).

To prepare nano-cochleates with tobramycin, procedures of Example 2 were followed, with the TAS solution replaced by the tobramycin solution of pH=2.5. The images of nano-
25 cochleates under an optical microscope is shown in Figure 6A. Similarly, addition of EDTA

caused conversion of nano-cochleates to small liposomes (Figure 6B). The particle size distribution of the nano-cochleates was measured using a Nicomp submicron particle sizer, showing the mean dynamic size was around 300 nm (Figure 7).

5 **Example 4. Preparation of cochleates with polylysine**

Cochelates can also be prepared by adding a solution of peptide into the liposomal suspension as in Example 1. When a solution of polylysine (MW = 1000 ~ 2000, pH =4) was added drop wise to the same liposomal suspension as in Example 1, precipitates were formed. The final ratio
10 of DOPS and polylysine was 1:1.2. A microscopic image showed that the precipitated particles possess a needle shape (Figure 8A). These needle shape particles readily opened up and converted to giant liposomes (Figure 8B) as those prepared with other bridging agents (Ca²⁺[11], Zn²⁺[11], TAS, and tobramycin).

15 **Example 5. Preparation of nano-cochleates with polylysine**

Nanometer sized cochleates can be prepared with peptides as the bridging agent in a way without using the hydrogel-isolation [11]. In this experiment, the liposomal suspension prepared as in Example 1 was added into the polylysine solution as in Example 4 under stirring
20 with the final lipid to polylysine ratio of 1:4. The clear liquids (polylysine solution and liposomal suspension) readily turned to cloudy. No visible particles were observed under optical microscope. A particle size measurement was carried out using a Nicomp Submicron particle sizer, suggesting the mean dynamic size of the particles was about 60 ~ 100 nm.

The mechanism of the size reduction due to increased polylysine to liposome ratio may be
25 similar to that in the complexation between DNA and cationic polymers [14].

Example 6. Loading capacity of cochelates for molecules as bridging agents.

Loading capacities of the new cochleates and nano-cochleates for TAS and tobramycin were determined.

For TAS, known amount of cochleates or nano-cochleates were first dissolved with chloroform,
5 then added with water (pH=2), followed by strong shaking. The water phase was separated here after, and the same procedure was repeated two more times. The water phases collected three times were combined. The concentration of the extract was determined by UV absorption at 380 nm.

10 For tobramycin, since the molecule is UV inactive, a HPLC method based on a reaction with a dye (a USP method) was used. The loading amount was determined based on concentration decrease in the supernatant after cochleate formation.

For mobile phase, 2.0g of tris (hydroxymethyl) aminomethan was first dissolved with 800ml of
15 water, followed by addition of 20ml of 1N H₂SO₄ and acetanitrile to a total volume of 2000ml.

For the detectability, a solution of 2,4-dinitrofluourobene (10mg/ml in alcohol) was prepared within 5 dyas prior to use and refrigerated. A water solution of tris (hydroxymethyl) aminomethane (15 mg/ml) was also prepared as a stock solution. Within 4 hours of analysis,
20 this stock solution, 40 ml, was diluted with dimethyl sulfoxide (DMSO) to 200ml.

For the standard curve, 550 mg of tobramycin and 2ml of 1N H₂SO₄ were and dissolved in water to make a solution of 100 ml in volume. Prior to analysis, this solution was further diluted by 5 times with the tobrmycin content 0.22mg/ml of tobramycin.

25

For the supernatant (the sample to be measured), 1ml of 5.5mg/ml of tobramycin solution was added into a liposome suspension with lipid to drug ratio of 1:5. After precipitation, the supernatant was collected and diluted to 50ml with water.

The standard and sample (the supernatant) solutions of tobramycin, each 2 ml, were added with 5 ml the pre-prepared 2,4-dinitrofluorobenzene solution and 5 ml the pre-prepared tris (hydroxymethyl) aminomethane solution. Then the standard and the sample were allowed to
 5 react with the added agents at 60 °C for 50 min. After cooling down, the two samples were diluted by acetonitrile to 25 ml.

Prior to HPLA measurement, the treated standard and the sample were further added with an acetonitrile solution of P-naphtholbenzein, respectively, at the ratio 4:1. HPLC analysis was
 10 carried out using a C-18 column with absorption selected at 266 nm.

The loading capacity for TAS and tobramycin are listed in Table 1.

15 **Table 1. Loading capacity of cochleates for 2,3,5,6- tetraaminopyrimidine sulfate and tobramycin.**

Model Drug	Lipids (mg)	Drug (mg)	Drug/Lipid (mole/mole)
TAPS*	5	0.0248	1.0 / 2.0
Tobramycine	38.11	5.5	1.0 / 4.0

Example 7. Antibiotic activity of tobramycin loaded in cochleates and nano-cochleates.

Tobramycin was selected as a model drug to examine the capability of cochleates and nano-cochleates to delivery hydrophilic drugs across cell membranes for that the antibiotic function of tobramycin relies on its binding to ribosomes inside of cells. In another word, for tobramycin, its antibiotic activity reflects internization of the drug into the cells. For this purpose, tobramycin loaded cochleates and nano-cochleates prepared as in Example 2 were incubated with E.Coli at various dose. As a control, a tobramycin solution was also incubated with E.Coli under identical conditions.

Experimentally, one drop of E.Coli cell line, DH5a, was added to 2 ml LB Broth solution and incubated at 37 °C for 24 hrs to prepare 1:10 DH5a culture solution. Then, 5 ul of the DH5a culture solution was diluted to 2 ml and added with tobramycin in the forms of cochleates, nano-cochleates at the final concentration of 0, 0.5, 1.0, 2.5 and 5.0 ug/ml, respectively. The tobramycin-added cell cultures were incubated at 37 °C, with shacking at 200 rpm, for 24 hrs. The incubated cell culture suspension were then diluted by 1:100, 1:1000, and 1:10000 times, and plated as 50 ul diluted cultures to each agar dish. The dishes were further incubated at 37 C for another 24 hrs prior to counting of the colonies. The result is shown in Figure 9.

At low doses (0.5 µg per 1 ml of medium, i.e. 0.5 µg/ml), the drug solution showed highest activity as that it was 75 times higher than that of large cochleates and slightly higher than nano-cochleates. With the dose increased to above 1 µg/ml (the dose suggested by USP), nano-cochleates became the most active dosage form. The cell counts for nano-cochleates treated culture was ten times lower than that by tobramycin solution and 100 times lower than that of large cochleates. At the dose of 5.0 µg/ml, cell counts became zero for all the three formulations. It is clearly that nano-cochleates significantly enhanced antibiotic activity of the drug. Based on loading capacity of the tobramycin and the average size of nano-cochleates, approximately 2,000,000 drug molecules are loaded in one nano-cochleate. Therefore, at the same tobramycin dose, the frequency for nano-cochleate particles to collide the cells should

be much lower than that of free drug molecules in the solution. Nevertheless, more drug molecules entered E. Coli cells in the nano-cochleate form as compared with the form of free solution (Figure 9). This result supports our hypothesis that the cochleate structure facilitated permeation of hydrophilic agents across cell membranes. Probably the high tension at the edge of the phospholipid bilayer at the two ends of cochleate cylinders facilitated fusion of cochleates with cell membranes (Figure 2).

At low dose (0.5µg/ml), the drug solution showed slightly higher activity probably due to that the number of nano-cochleate particles was too less for sufficient exposure of the cells (to the drug). The same reason also explains the relatively lower activity for large cochleates (Figure 9).

The properties that nano-cochleates can facilitate cross-membrane diffusion for charged and impermeable molecules have a wide application in drug delivery. Many therapeutic agents, such as peptides, are soluble but impermeable to tissue membranes. Cross-membrane permeation is especially important for those agents for which the binding sites are inside of cells rather than cell surface receptors. The system may also facilitate oral absorption for peptide drugs that possess net positive charge.

The nano-cochleates demonstrated in the inventor's previous invention [11] showed significant in vivo bioavailability and therapeutic efficacy for oral delivery of amphotericin B, a hydrophobic anti-fungus agent normally administrated through IV route. The new nano-cochleates, although differing from the previous one by using organic cations as the bridging agents, possess similar physical chemical properties including the ability of fusing with cell membranes (Example 7). Therefore, the new system is expected to have the ability to deliver impermeable therapeutics orally.

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What is claimed is:

1. A cochleate system, wherein the agents bridging lipid bilayer are organic multi-valent cations.
- 5 2. The cochleate system of claim 1, wherein the cochleate is a nano-cochleate.
3. The cochleate system of claim 1, wherein particles may or may not form cylindrical shapes depending on the size of organic cations.
- 10 4. A method for preparing the cochleate system of claim 1, comprising direct cochleation and hydrogel-isolated procedure.
5. A method for preparing the cochleate system of claim 1, comprising control of the cochleate size by the ratio of poly-ionic bridging agents to lipids.
- 15 6. The method of claim 5, wherein the size of cochleate is about 40 nm to about 1000nm in dynamic diameter.
- 20 7. The cochleate system of claim 1 for microencapsulation and delivery of therapeutics, wherein the therapeutic agents are loaded in the cochleate structure as the bridging agents between lipid bilayers.
- 25 8. The cochleate system of claim 7, wherein the therapeutics are selected from a group consisting of peptides, poly-amino acids, nucleotides and hydrophilic chemical drugs which possess more than two net positive charges under the condition of preparation.
9. The system of claim 1 for delivery of tissue-impermeable or hydrophilic therapeutics.

10. The system of claim 1 for oral delivery of peptides.

11. The system of claim 1 for delivery of therapeutics through inhalation.

5

12. A composition comprising the system of claim 1.

13. A pharmaceutical composition comprising the system of claim 1 and a
pharmaceutically acceptable carrier.

10

14. A method to treat a subject with a disease comprising administering to the subject the
system of claim 1 which comprise an appropriate drug for said disease.

15. A method to adjust the inter-bilayer distance of cochleates, comprising using designed
organic bridging agents with defined molecular size.

15

16. The method of claim 15, wherein the inter-bilayer distance is from a few angstrom to a
few nanometers.

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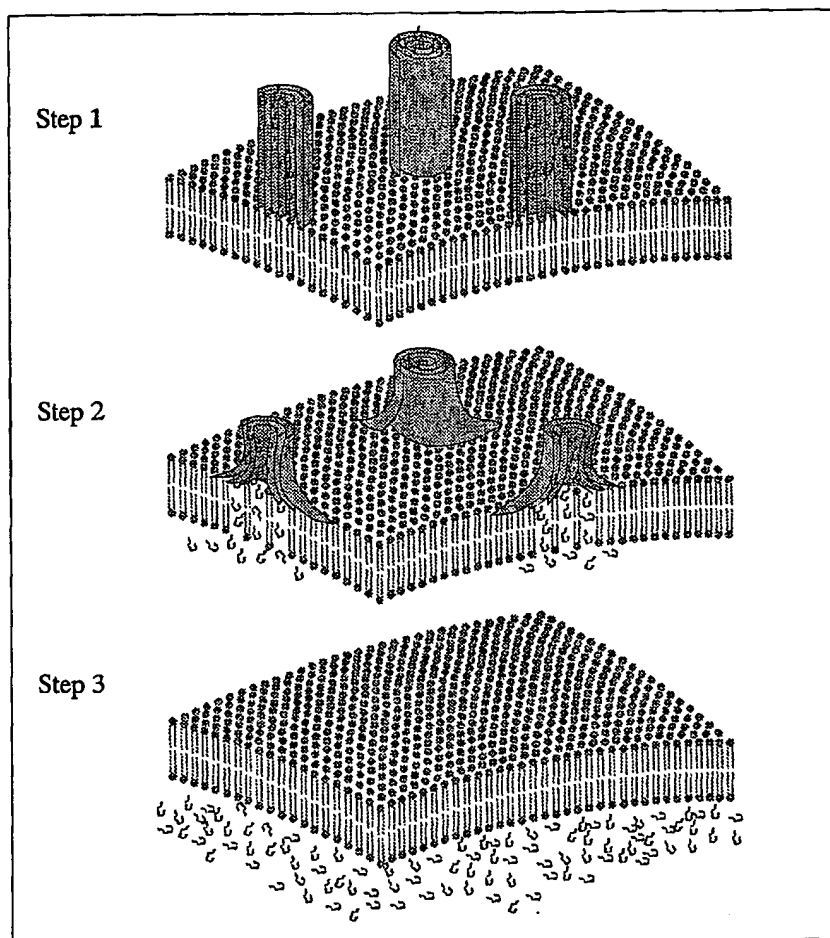
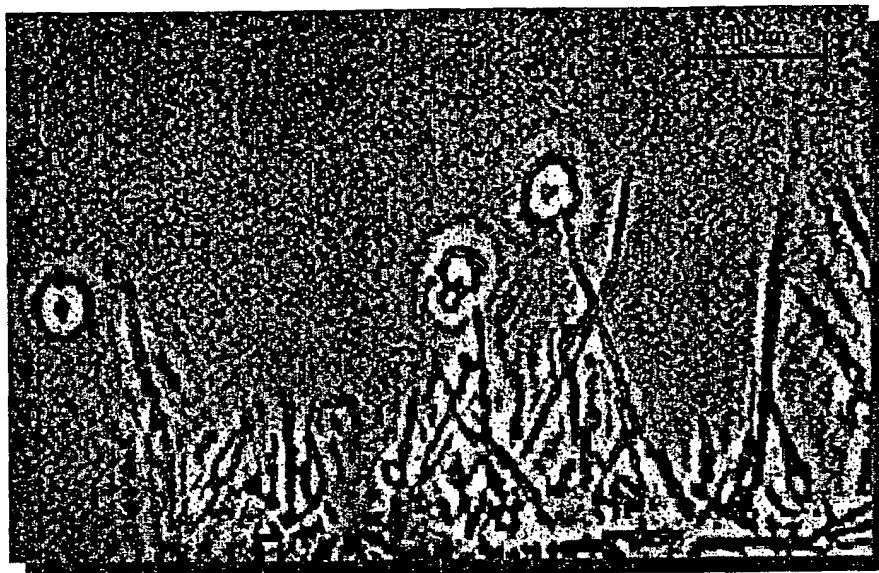


Fig.2

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A



B

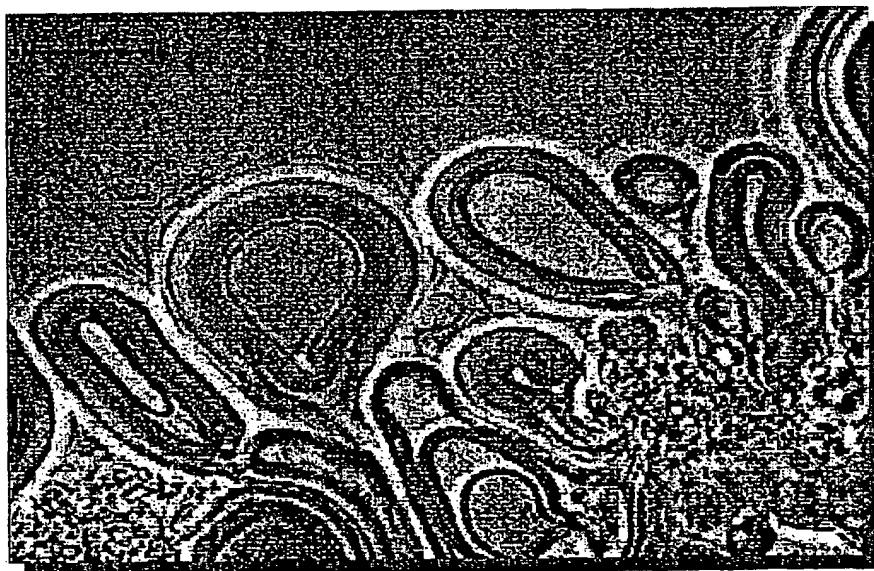
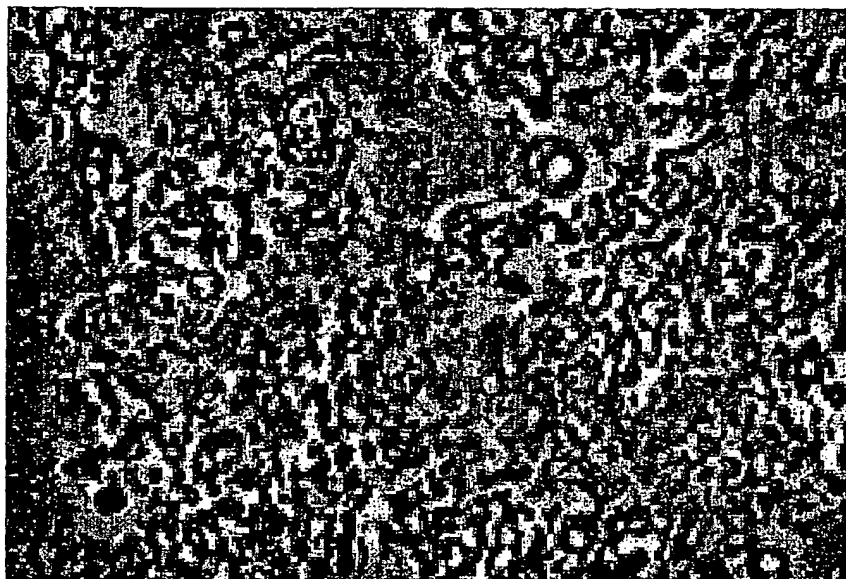


Fig.3

4/9
A



B

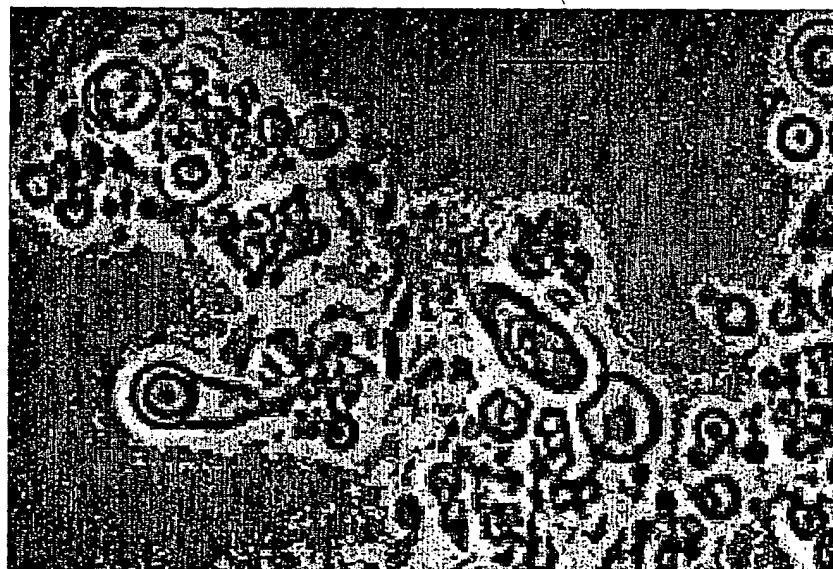


Fig.4

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A



B

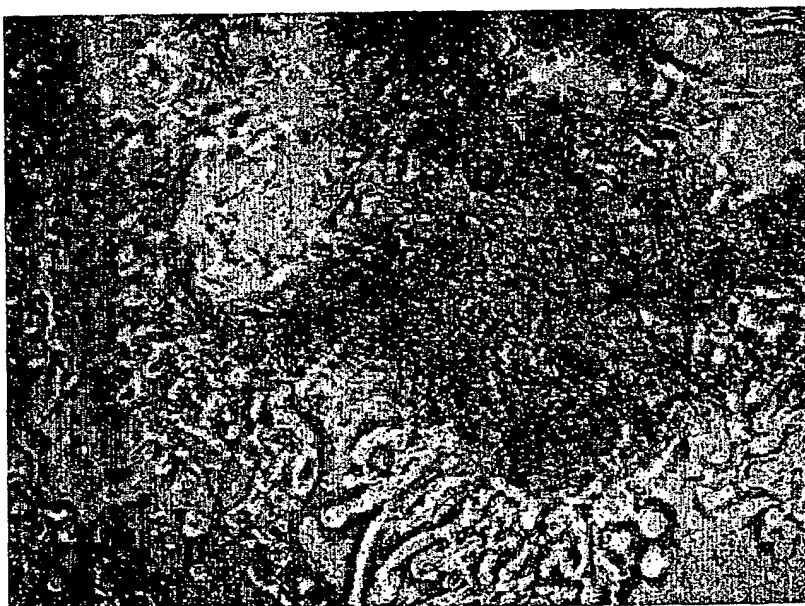
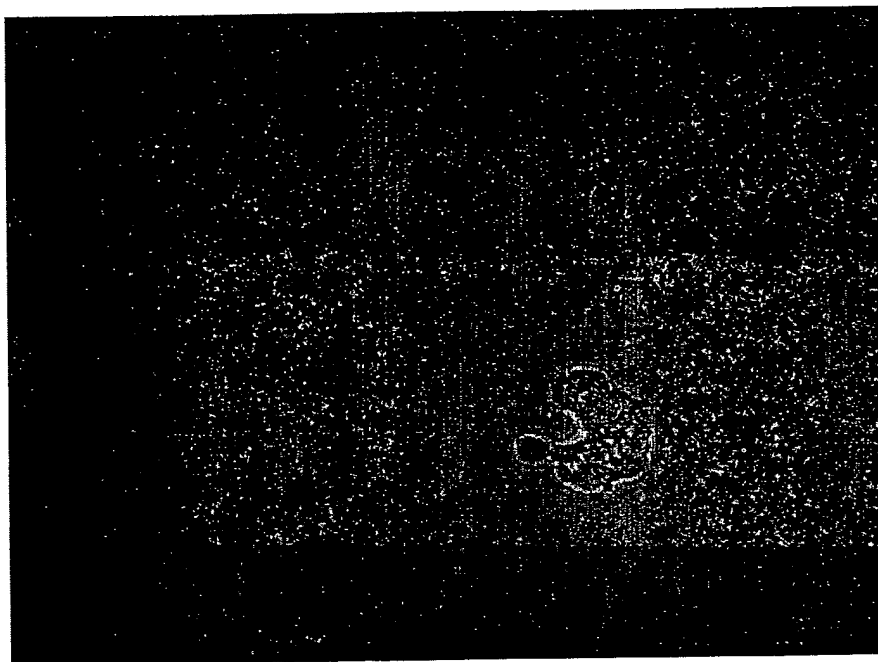


Fig.5

6/9
A



B

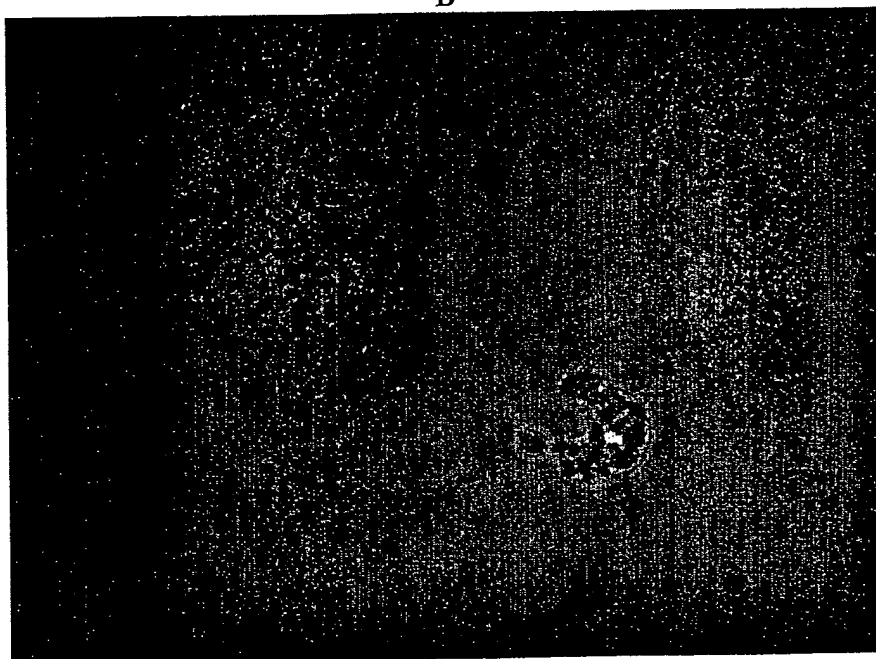


Fig.6

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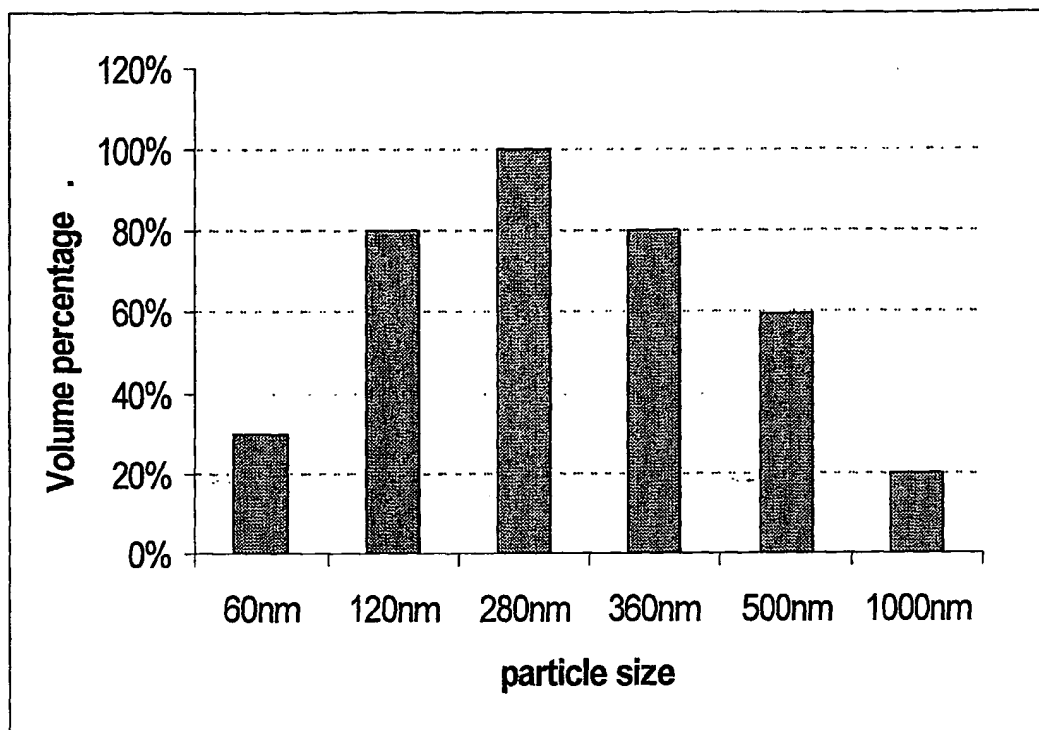
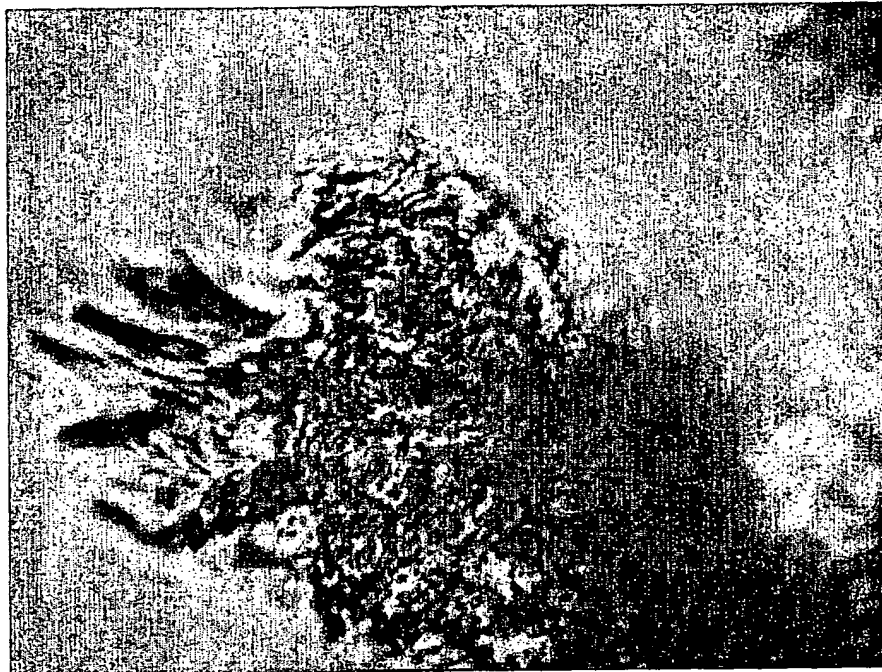


Fig.7

8/9
A



B

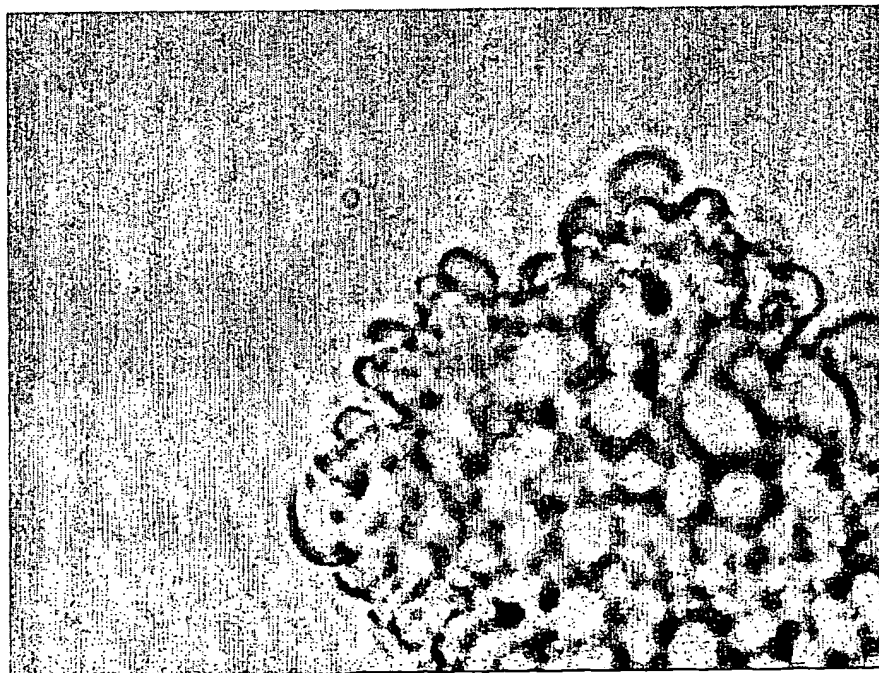


Fig.8

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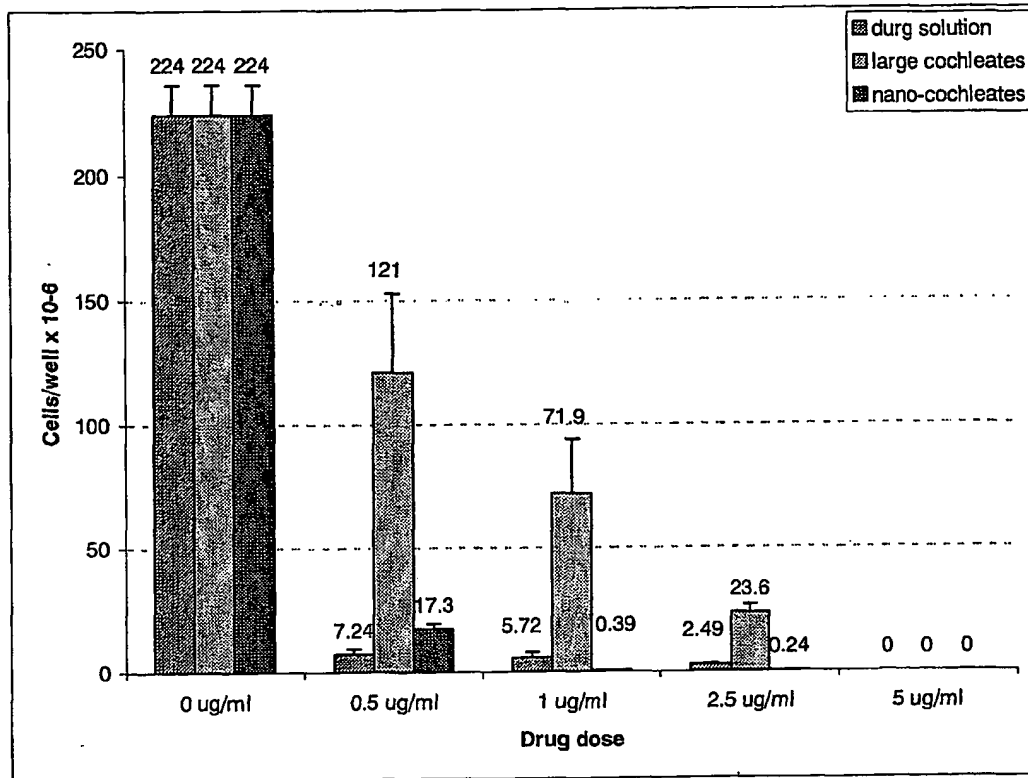


Fig.9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN03/00634

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁷:A61K9/127,48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Chinese patent

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI;EPODOC;PAJ;CPRS;CA;CNKI Search words:cochleate,ect.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,5994318(Albany Medical College)30.Nov.1999(30.11.99),whole	1-16
A	WO,A2,0035421(UNIVERSITY OF MARYLAND)22.Jun.2000(22.06.00),whole	1-16
A	US,A,6153217(Biodelivery Sciences,Inc.)28.Nov.2000(28.11.00),whole	1-16
A	US,B1,6340591(University of Maryland)22.Jan.2002(22.01.02),whole	1-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
31.Oct.2003(31.10.03)

Date of mailing of the international search report
27 NOV 2003 027.11.03

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